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BIOLOGICAL BULLETIN

THE LIFE HISTORY OF DESMOGNATHUS FUSCA.

(*Continued from March Issue.*)

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THE AQUATIC LARVAL PERIOD.

Following the brief but very important terrestrial larval period, the aquatic larval period begins. As may be computed from the limits of dates during which egg-laying occurs (June 1 to Sept. 1), the probable duration of incubation (5 weeks), and the somewhat variable period of terrestrial life (1 to 2 weeks), the date of the beginning of the aquatic period can hardly be earlier than the middle of July or later than the middle of October. I have personally never known of aquatic larvæ being found between June 17 and September 1. By September 15 they are present in the brooks in great abundance, the majority of them with yolk still present in the intestinal walls, a condition not unlike that shown by stage *F* of the terrestrial larvæ and never as yet found by me in specimens collected in October. These facts point to early September as the time when the majority of the larvæ reach the water and begin their aquatic existence. The wide range of variation in the size and proportions of larvæ during September and October (cf. Table II. and Graphs III. and IV.) represents the inevitable difference in age resulting from the long period of egg laying.

As to the duration of the larval period, we have the statement of Reed and Wright ('09): "The larvæ transform from September to December, when they are from 18 to 20 mm. long." My own observation does not confirm this statement. It is a very significant fact that after the larvæ once appear in the water in September, they are found about equally abundantly

during the fall, winter, and early spring months, and that, as shown by Table II. and its accompanying Graphs III. and IV., the average proportions and size of the body remain practically the same until late spring, when there occurs a marked increase in average size and a decided change in the proportionate lengths of the regions of the body in that the tail lengthens more rapidly than either the head or trunk. Structurally, also, the specimens taken during the fall and winter display none of the indications of approaching metamorphosis which are so unmistakable when they do appear in the May specimens. Moreover I have never found larvæ actually undergoing metamorphosis except in June, nor any of the very small recently metamorphosed adults except in June and the early part of July. The aquatic larval period would thus appear to extend practically through autumn, winter,

Table II.

Aquatic Larval Period.

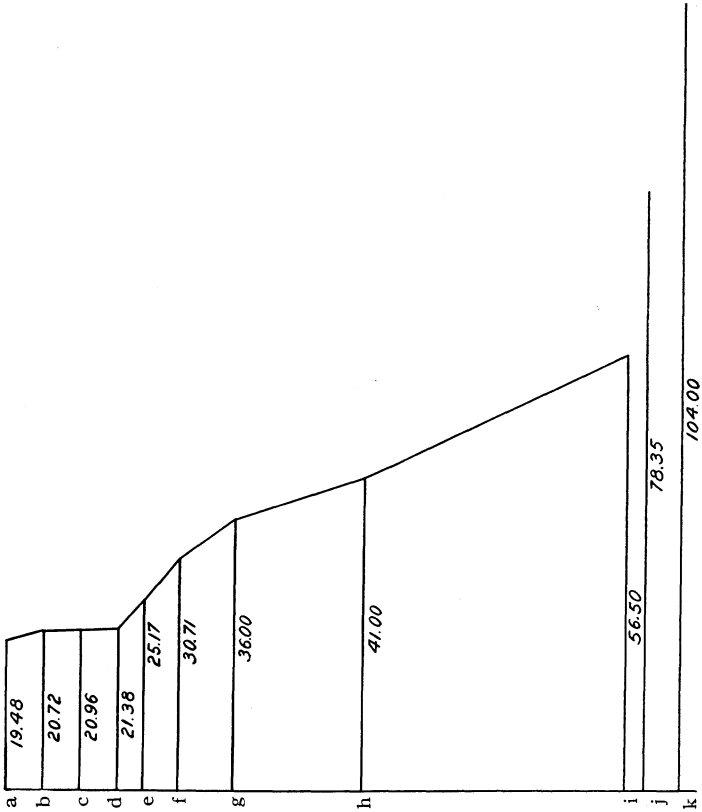
When Collected.	Number of Examples.	Column I. Actual Sizes (Cf. Graph III.), Mm.			Column II. Proportionate Lengths of Body Regions (Cf. Graph IV.).		
		Maximum.	Minimum.	Average.	Maximum.	Minimum.	Average.
September and October.	42	Head 5.00	4.00	4.34	.208	.261	.223
		Trunk 7.25	4.30	5.86	.302	.281	.301
		Tail 11.75	7.00	9.28	.489	.458	.476
		Total......24.00	15.30	19.48	1.000	1.000	1.000
November and December.	15	Head 5.40	4.00	4.56	.212	.234	.220
		Trunk 7.60	5.50	6.38	.298	.324	.307
		Tail 12.50	7.50	9.62	.490	.441	.473
		Total......25.50	17.00	20.72	1.000	1.000	1.000
January and February.	5	Head 4.55	4.25	4.51	.207	.224	.215
		Trunk 6.83	5.75	6.45	.311	.303	.308
		Tail 10.62	9.00	10.00	.483	.474	.478
		Total......22.00	19.00	20.96	1.000	1.000	1.000
March and April.	8	Head 4.50	4.10	4.395	.191	.217	.206
		Trunk 7.50	5.20	6.655	.319	.298	.311
		Tail 11.50	8.80	10.335	.489	.485	.483
		Total......23.50	18.10	21.385	1.000	1.000	1.000
May.	5	Head 6.00	4.75	5.12	.182	.218	.203
		Trunk 10.00	6.75	7.55	.303	.310	.300
		Tail 17.00	10.25	12.50	.515	.471	.497
		Total......33.00	21.75	25.17	1.000	1.000	1.000

ADULT PERIOD.

When Collected.	Number of Examples.	Column I. Actual Sizes (Cf. Graph III.), Mm.			Column II. Proportionate Lengths of Body Regions (Cf. Graph IV.).		
		Maximum.	Minimum.	Average.	Maximum.	Minimum.	Average.
Metamorphic stage. Collected June 17 to July 1,	3	Head 5.60 Trunk 9.40 Tail 14.00	5.50 7.75 14.50	5.53 8.72 14.17	.193 .324 .493	.198 .279 .522	.195 .306 .501
		Total......29.00	27.75	28.42	1.000	1.000	1.000
Small adults. Collected June 5.	2	Head 6.70 Trunk 10.50 Tail 17.80	6.50 10.30 16.50	6.60 10.40 17.15	.191 .300 .509	.195 .309 .496	.193 .305 .502
		Total......35.00	33.30	34.15 Average of metamorphic and smallest adults 30.71	1.000	1.000	1.000
Small adult. Collected Sept. 17.	1	Head.......... Trunk......... Tail...........	....	6.50 10.50 19.00	....	....	.181 – .292 – .528 –
		Total..........	......	36.00	......	......	1.000
Small adult. Collected May 3.	1	Head.......... Trunk......... Tail...........	....	7.00 12.50 21.50	....	....	.171 .305 .524
		Total..........	......	41.00	......	......	1.000
Miscellaneous set of adults. Collected in June and July.	10	Head 16.00 Trunk 31.00 Tail 57.00	9.00 16.50 31.00	12.35 23.65 42.35	.154 .298 .548	.159 .292 .549	.158 .302 .540
		Total.....104.00	56.50	78.35	1.000	1.000	1.000

It will be noted that in the above table the number of examples collected during September and October is greatly in excess of the number collected during the other months of the aquatic larval period. This fact is not due, as would seem to be indicated, to a greater abundance of the material during these months, but rather to the fact that these happen to be the months when most of my collection of *Desmognathus* material for laboratory purposes is made.

and spring, and to cover a somewhat variable period of from 8 to 10 months. The perennially running brooks in which they live are of course spring fed and do not freeze solid even though they are very shallow. In fact the brook which I have particularly studied as a typical *Desmognathus* habitat is said to never



GRAPH III., showing the growth in length during the aquatic larval period (lines *a–f*), metamorphosis (line *f*), and the first two years of adult life (lines *f–i*); also, for comparison with these, the average length (line *j*) and the maximum length (line *k*) of ten miscellaneous adults. Based upon the statistics given in the first column of Table II.

The time ratios are indicated by measurements horizontally. The vertical lines show, natural size, the average lengths of specimens as follows:

Aquatic larval period:

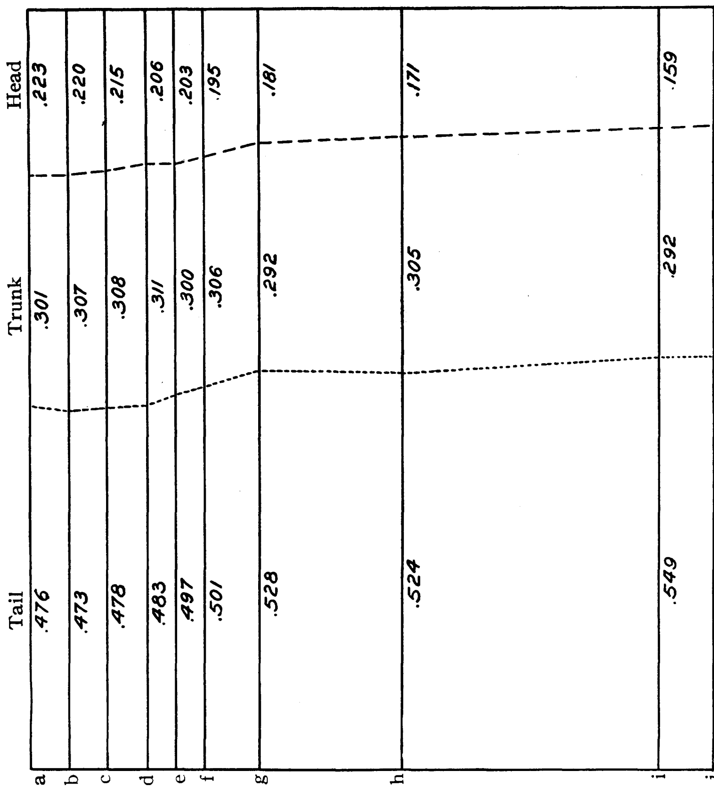
a, collected in September and October.
b, collected in November and December.
c, collected in January and February.
d, collected in March and April.
e, collected in May.

Metamorphosis:

f, collected in June and July.

Adult period:

g, collected in the September following metamorphosis.
h, collected in May nearly one year after metamorphosis.
i, collected in June and July two years after metamorphosis.
j, average of 10 miscellaneous adults.
k, maximum of 10 miscellaneous adults.



GRAPH IV., showing a comparison of the proportionate lengths of body regions during the aquatic larval period (lines *a–f*), metamorphosis (line *f*), and the first two years of adult life (lines *f–i*); also the proportionate lengths of body regions of the average of 10 miscellaneous adults (line *j*). Based upon the statistics given in the second column of Table II.

The time ratios shown by the horizontal measurements and the dates expressed by the location of the vertical lines are as in Graph III. Note that by the end of the second year of adult life (line *i*) the body proportions are those of the average adult (line *j*).

entirely freeze over. Thus I have always found, even in midwinter, a little open space, sometimes only a few inches in width, and by breaking away the ice bordering the edges of this, have been able to reach the quiet pools under the ice, in which larvæ are found. Specimens have in this way been collected in January when the temperature was at 0° F. and had been at that point or lower for three or four days.

The larvæ frequent the quieter regions, always out of the current except when a temporary disturbance drives them into it. They seek not only quiet but very shallow water, where they lie motionless among the decaying leaves, which, owing to the wooded nature of their habitat, fill such pools. Such leaves also furnish quiet lurking places when they lie in a thick mass covering the surface of the more rapidly moving regions of the stream. Among the loose debris and sediment which these surroundings afford, the larvæ lie, their mottled brown color forming a perfect protective resemblance. The collector may thus at first stare several minutes into such a little pool before he recognizes in an apparent fragment of the midrib of a decaying leaf, the mid-dorsal ridge of a *Desmognathus* larva, and he may possibly realize the true nature of the object under observation only when upon disturbing the water, active swimming movements betray the living animal. On the other hand having once begun to recognize the larvæ one is frequently deceived into picking up some lifeless fragment which the larvæ so closely resemble. Several larvæ are frequently found in close proximity in the same little pool or mass of leaves, a fact which probably results from the abundance of the larvæ, but may in the early fall be due to the simultaneous arrival, in the same little pool, of several individuals of the same brood.

Frequently the larvæ, when concealing themselves among the leaves, lie half out of the water, the body immersed just sufficiently to bring the gills under the surface. This habit I have observed particularly in the case of individuals in captivity, and have thought that it might be associated with lack of sufficient oxygen. However the larvæ are certainly not adapted to the pressure conditions of deep water, and although they are quiet and apparently comfortable when in captivity in water a centimeter deep, if the depth of the water be gradually increased they show signs of uneasiness when it becomes about two centimeters, and by the time a depth of three or more centimeters is reached they make frantic efforts to reach the top of the water, where, however, they can sustain themselves only by active swimming or by resting upon some surface. In fact under these conditions they almost invariably swim to the edge of the aquarium, run

rapidly up its side, and attempt to make their escape, while in shallow water they remain for days in an open dish and make no such attempt. This adjustment to shallow water is undoubtedly a means for insuring that they remain in regions where at the time of metamorphosis they can reach the necessary terrestrial environment; for the adults, being lungless, have no hydrostatic organs, neither have they any method of aquatic respiration other than the skin.

The larvæ react vigorously to attempts to capture them by any seizing act or any method which involves pressure against the opposite sides of the body, being evidently organized to escape from jaws and teeth. That they frequently barely succeed in making such escapes is evidenced by the large proportion of maimed individuals which one finds, tails and hind legs being the parts most frequently lost. The location of the lateral line organs or neuromasts upon opposite sides along the lateral surfaces of the body may have some significance in connection with this reaction. On the other hand, the larvæ do not appear to be especially sensitive to mere tactile stimuli even when these are applied to the regions where the neuromasts are most abundant, unless such stimuli are applied upon two opposing regions. After the application of such opposing stimuli, however, or the repeated application of single stimuli, the sensitiveness of the larva to touch seems to be for a time considerably augmented, and a stimulus applied to a single region will then give rise to vigorous efforts to escape.

On the other hand the larvæ appear to have no reflex mechanism for protection against forces which gently raise and lower the body, like the slight natural movements of quiet water. They may therefore be readily captured by lifting them with the hand, a watch glass, or any other concave object which may be passed gently under them and then quietly raised; the gradual flowing of the water from the surface upon which they are thus lifted produces also no disturbance so long as the larva remains moist. Larvæ may in this way be transferred from their natural habitat to the collecting jar, without a single movement on their part, while the slightest pinching or seizing force results almost invariably in such violent wriggling that the animal makes its escape.

The food of larvæ during the aquatic period consists mainly of little copepods and occasionaly very small aquatic insect larvæ. Mingled with the debris of such food in the stomach and intestine, there are usually found considerable quantities of sand and disintegrating vegetable matter, the ingestion of which is incident to the capture of the living food. The mouth opening does not extend very far back and it is equipped at each angle with a labial fold (*lf*, Fig. 17) so arranged that when the lower jaw is depressed the folds are stretched across the angles making the orifice almost circular in form and directed slightly downward. This is evidently an adaptation to the capture of such food forms as may be scooped up or sucked in from among the sediment at the bottom or upon the surfaces of decaying leaves.

The respiration during the larval period is accomplished by means of the three pairs of external gill bushes. Although these consist of relatively few filaments, they are held so widely outspread in the water as to render their position the most advantageous one possible to the performance of their function. When the larva is disturbed, however, as by opposing pressure applied to the lateral surfaces of the head, the gills are quickly drawn back and held closely appressed against the sides of the body, a protective reflex the efficiency of which is shown by the fact that one never finds larvæ with the gills injured.

With four gill slits upon each side, it would seem that the mechanism must be present for producing a flow of water through the mouth and pharynx and out over the gills, yet I have never been able to detect the slightest evidence of such a current, or of the rhythmic movements of the floor of the mouth and pharynx such as would be necessary to produce it. Occasionally there may be observed, however, a single, vigorous little movement of the gill bushes, which is undoubtedly for the purpose of assisting diffusion by hastening the change of the water in contact with the gills. This movement is usually performed once or twice when the larva comes to rest after vigorous swimming. The usual position of the larvæ when at rest, upon or among decaying leaves just below the surface of the water, gives natural access to the region of the water which is most completely aerated.

The gill filaments present a certain glistening white appearance

which at times renders them the most conspicuous portions of the body. This appearance is similar to certain white spots which occur along the lateral surfaces of the body and occasionally in other regions, in both the larval and the adult stages, and have been described as "white pigment spots." There is considerable evidence, however, that in the gills, at least, the phenomenon is due to an accumulation of some gas, presumably carbon dioxide, in the tissues, but its exact nature and significance demand further investigation.

In the matter of internal structure as in size, the aquatic larva appears to be in a general static condition throughout the larger part of its larval life. Late in the spring, however, shortly before the metamorphosis, a series of changes becomes inaugurated, involving especially the integument and the organs derived from it, as well as certain skeletal parts, and leading rapidly to the conditions which characterize the adult. Such changes are to be considered as belonging particularly to the period of metamorphosis, and will be described as such.

Until such distinctive metamorphic changes appear, the skin of the larva remains in practically the condition attained at the end of the terrestrial period. The two layers of cells of the epidermis remain fairly well defined. The cuticular border of the outer layer becomes thicker and more compact in appearance, and the cap of intracellular pigment between this border and the nucleus becomes denser. In the deeper layer, the cells of which rest directly upon the dense corium, the distended vacuolated Leydig cells are in most regions so abundant that each is usually separated from the neighboring Leydig cells by but a single circle of cells of the general epidermal type (Plate IV., 25). Here and there among the cells of the deeper layer are cells which are considerably elongated and somewhat piriform and which extend out between the cells of the outer layer almost to the external surface. These are the cells which in the German literature are designated as the "Schaltzelle" (Plate IV., 23, *intr*). As the larval period advances there is a gradual increase in the thickness of the epidermis due to the increasing size of the cells of the deeper layer, especially the Leydig cells, which gradually become more distended, while their nuclei become notice-

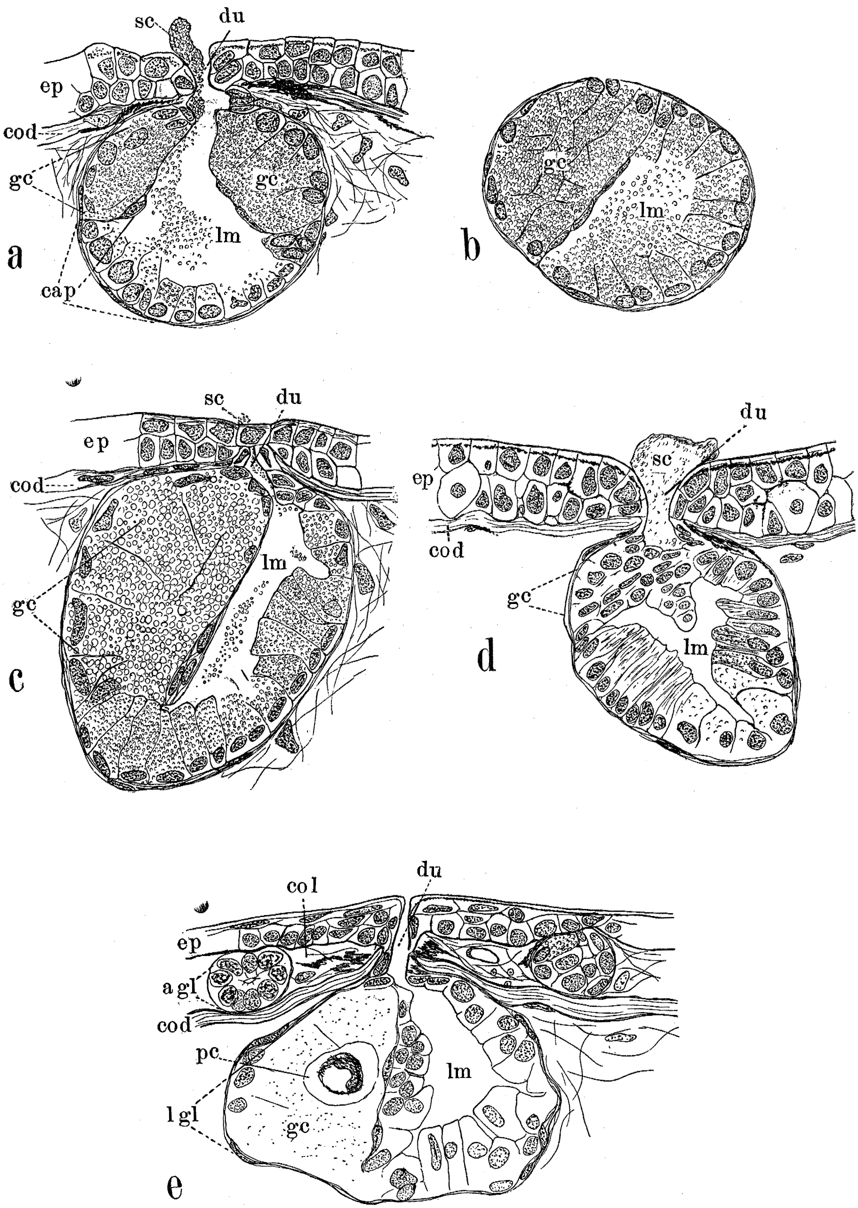


FIG. 15. Sections through larval acinous glands; (*a*) vertical section, *i. e.*, at right angles to the external surface, through the middle of a gland of the supra-branchial group of a recently hatched terrestrial larva; (*b*) cross section, *i. e.*, parallel with the external surface, through the middle of one of the mid-dorsal

ably smaller. The nuclei of the cells of both layers appear in many cases irregular in form and often show sharp constrictions which suggest amitotic division, a suspicion further confirmed by the increasing number of multinucleate cells which, in spite of the absence of mitotic phenomena, appear in the outer layer of the epidermis, particularly in the latter part of the larval period. As the Leydig cells become larger with the continuance of larval life, some of the surrounding cells of the deeper layer become crowded together into a columnar form, while others appear to be pushed out of line, some lying in the deeper region of the epidermis and others becoming crowded into the angles between the bases of the cells of the outer layer (Plate V., 29). Thus the number of cells which reach from the deeper layer into the outer layer becomes increased. These, however, seem not to actually belong to the external layer since, even if they reach the surface, they apparently do not acquire a cuticular border. These slight changes in the epidermis are accompanied by a gradual increase in the thickness of the dense corium, from which, moreover, connective tissue cells gradually invade the epidermis. These together with leukocytes and branches of nerve fibers may thus be seen among the cells of the deeper layer, and not infrequently these invaders, as well as the pigment cells, actually break through the thin membrane of the Leydig cells, and lie within the large vacuolated spaces which these afford.

The acinous glands (Fig. 15), which have already developed and begun to function at the time of hatching, remain in a fully

group of glands of a terrestrial larva; (*c*) vertical section through one of the glands of the supra-branchial group of an aquatic larva in late spring, hence near the end of the larval period; (*d*) vertical section through one of the supra-branchial group of an aquatic larva in February, showing an almost completely discharged condition induced by prolonged mechanical stimulation immediately before the specimen was killed; (*e*) vertical section through one of the supra-branchial group of an individual immediately after metamorphosis, showing the gland in an empty condition and undergoing atrophy, while in the loose corium nearby are seen two of the newly developed acinous glands of the adult period of life. Note the position of the larval gland below the level of the dense corium, while the adult glands are in their permanent location external to this layer.

Agl, adult gland; *cap*, capsule of the gland; *cod*, dense corium; *col*, loose corium; *du*, duct of the gland; *ep*, epidermis; *gc*, giant cells of the gland; *lm*, lumen of the gland; *pc*, pigment cell which has invaded the gland; *sc*, secretion discharged from the gland. Drawn with Abbé camera. × 225.

active condition throughout larval life. These glands, arranged as above described (cf. p. 291 Vol. XXIV. *Biol. Bull.*), lie quite below the dense corium, embedded in the subcutaneous connective tissue, and communicating with the external surface by means of a narrow duct. Although of the type of amphibian gland variously designated as "granular" or "poison," they do not in detail correspond in all respects to the descriptions of such glands in other amphibians. They vary greatly in size, the largest when in a distended condition having a diameter eight times the thickness of the skin, while the smallest when distended have a diameter only twice the thickness of the skin. They are spherical or subspherical in external form; the larger ones, especially those of the suprabranchial group, are not of the simple acinous type which this external form suggests, however, since internally the lumen is partially subdivided by invaginations of the single layer of gland cells, as well as by the occurrence here and there of groups of these cells which are much taller than the rest and thus encroach upon the lumen. The connective tissue sheath (*cap*) of the gland follows closely the invaginations. The glands of all sizes are particularly characterized by the development of certain cells into the "giant" type (*gc*) through an enormous accumulation of granules of the same sort apparently as those which fill the other cells of the gland. These giant cells are differentiated very early in the development of the glands, even while mitosis is still in progress and before the lumen of the gland has appeared; in all of the glands they comprise at least one group of some eight or ten cells which lie near the orifice of the gland upon one side and become almost completely surrounded and cut off from the rest of the gland by the ingrowth of the connective tissue sheath (Fig. 15, *b*). Thus placed they form a mass nearly equal in volume to half of the whole gland, so that a section through the middle of the gland parallel with the external surface of the skin often shows the remainder of the gland wrapped in a crescentic form about this mass of giant cells. Giant cells occur also in other parts of the larger glands, either as isolated cells or in small groups of two or three. The granules filling all of the gland cells are similar, except that those in the giant cells become much larger. The ordinary cells, however, appear to constantly dis-

charge their contents into the lumen of the gland, where they form into a fluid which may often be seen in the sections in the process of being discharged through the gland duct. The discharge of the granular contents of the gland cells seems to involve a rupture of the cell membrane. The giant cells, on the other hand, seldom appear to be discharging their granules even when the remainder of the gland shows great activity, although the membranes of a group of such cells not infrequently appear to be ruptured, and a large accumulation of granules to be ready to be discharged. When, however, a larva is killed after having been subjected to much mechanical stimulation in the nature of poking and seizing with the forceps, not only are the ordinary gland cells found empty or nearly so, but the giant cells also are empty and the mass of cells, separated from the rest of the gland by its sheath, is collapsed (Fig. 15, *d*). It thus seems that these giant cells are in the nature of a reserve supply, ready for instant response in an extreme emergency when the constant secretive action of the ordinary gland cells is inadequate. The only other possible explanation is that the secretions of the ordinary and the giant cells are different in function, a supposition which may upon further investigation prove to be correct, although the appearance of the secretions within the cells gives no indication of such a difference.

The condition of the nuclei of the gland cells in the fully discharged condition does not in the least indicate that the cells are doomed to degeneration; the nuclei are large and round and appear fully vigorous, much like the nuclei of the epidermal cells. Neither does it appear that there is any provision for the replacement of glands during the larval life, either in the form of single replacement cells or the anlagen of new glands; and, as all the glands, both large and small, are equally mature and active and exist in practically the same numbers throughout the whole larval life, there is no chance for the supposition that the small ones grow into the large ones. We are forced, then, to the belief that the larva hatches fully equipped with a set of glands of the granular type that are to perform their function without renewal throughout the many months of larval life.

That this function is important is very evident, though its

exact nature is a problem. The usual explanation of the function of the granular type of gland in the Amphibia is that its secretion is of a poisonous nature and protects the animal from capture by other forms which might use it as food. The full discharge of the contents of the glands by the *Desmognathus* larva under prolonged mechanical stimulation seems to corroborate this explanation, as does also the special supply of these glands in the region of those extremely important organs, the gills. On the other hand, the enormous development which this type of gland reaches in forms like *Bufo* and *Plethodon*, which have become very terrestrial, seems to indicate a correlation, at least, with some condition incident to terrestrial life, a conclusion further substantiated by the fact that the metamorphosis to the adult state in *Desmognathus* involves the development of a great multitude of glands of the granular type distributed over the entire surface of the body, in place of the few isolated groups of such glands with which the larva is supplied. Further, the fact that so closely related a form as *Spelerpes bilineatus* does not possess such glands while in the larval state leads one to ask what difference in environment or habits of these two species could serve to explain so decided a structural difference. We at once remember that the most essential difference lies in the fact that *Desmognathus fusca* is terrestrial in its egg laying habits while *Spelerpes bilineatus* is aquatic, and that consequently there is no terrestrial larval period in the latter species. Moreover, *Spelerpes* larvæ live in deeper pools than do the *Desmognathus* larvæ, and so far as I have observed, do not have the habit of lying upon leaves at the very surface of the water, which is so characteristic a position of the *Desmognathus* larvæ, and one which brings out of the water exactly those regions of the body which are supplied with the acinous glands, the mid-dorsal region of the trunk and the slightly elevated latero-dorsal surfaces of the head. Here again, then, there seems to be a decided connection between exposure to air and the function of the granular acinous glands. The solution of the problem is one which can be made only after extended experimental comparative study of different amphibians and must therefore be postponed until such study is completed.

The other set of specialized integumental organs, the neuro-

masts, are also prominent features of the skin during the entire larval life. They remain in practically the same condition except that as the skin becomes thicker and more compact, the accessory cells of the neuromasts become more numerous and more closely crowded together. Organs of this type are said to be associated with aquatic life and to be adapted to the reception of pressure stimuli. In view of this, it is interesting to note that the neuromasts are probably connected with the ready response of the larva both to a slight increase in the depth of the water above it, and to mechanical pressure applied to opposite sides of the body.

Metamorphosis.

Metamorphosis occurs, as above stated, in late spring. The earliest date at which I have collected larvæ showing signs of the approaching change is May 11, the latest is June 17. There is strong evidence, however, in the histological conditions and body proportions of the small adults of from 33 mm. to 35 mm. in length which I have collected early in June, that they have only recently undergone metamorphosis, and adult specimens of similar size and proportions may be collected in early fall also (Table II. and Graphs III. and IV.). It thus seems probable that the period of metamorphosis may begin as early as May and may extend well into the summer, a range of time comparable to that known to exist for both the egg-laying and the hatching periods.

Larvæ which are undergoing metamorphosis are usually not found in the water but among the dead leaves and loose debris along the edge, often headed away from the water as if they were in the act of crawling out of it.

The noticeable changes in external appearance which take place during the process are the following:

1. The body proportions change, the head becoming relatively shorter and its posterior region narrower and the tail longer.

2. The median fin fold characteristic of the aquatic larval period undergoes atrophy, the cross section of the tail thus acquiring a more rounded contour.

3. The gill bushes gradually diminish in length, each filament becoming blunt at its extremity instead of slender and pointed,

and finally the gills entirely disappear, a process involving the final closure of the gill slits.

4. The whole gill region becomes shorter and narrower, the latter change being due in part to the flattening down of the prominent bulging supra-branchial regions so that this portion of the head is no longer the widest one.

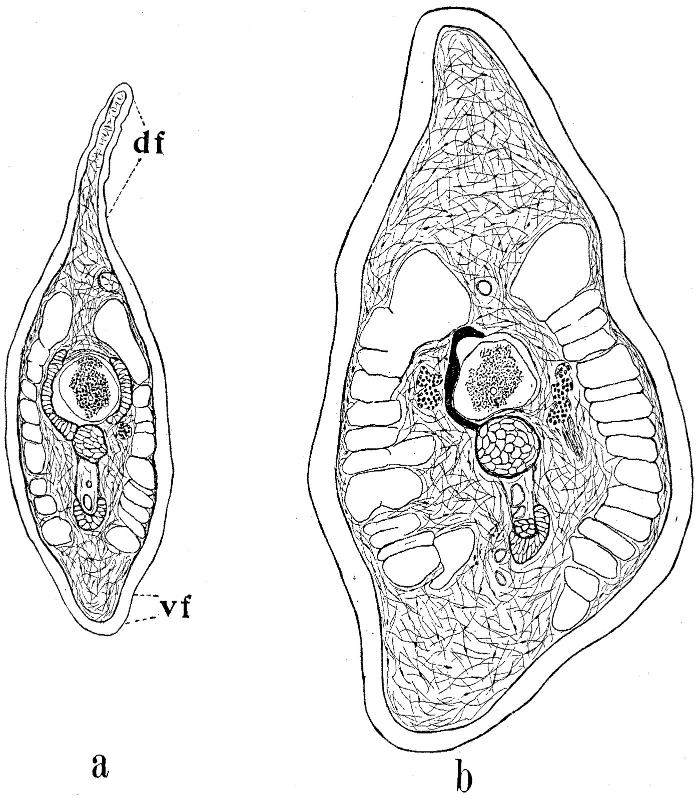


FIG. 16. Cross sections at the level of the 10th vertebra posterior to the cloaca showing (*a*) the typical aquatic larval form of tail with the dorsal (*df*), and the beginning of the ventral (*vf*) fin fold; (*b*) the change of form at the approach of metamorphosis. Note that (*b*) is cut slightly obliquely. Drawn with Abbé camera. × 60.

5. Further striking changes in the appearance of the head are due to the development of eyelids (*eyl*, Fig. 17) and of glandular masses in the orbit ventral to the eye, so that the eye and its surroundings come to possess the bulging appearance which is so characteristic an adult feature.

6. The naso-labial groove (*gr*, Fig. 17) makes its appearance, extending from the latero-ventral border of each external naris until finally, at the completion of metamorphosis, it has reached the border of the upper lip.

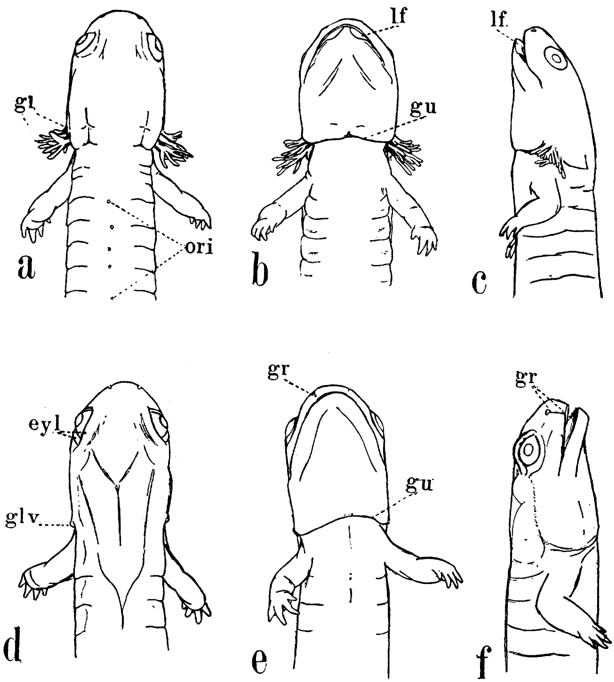


FIG. 17. (*a*), (*b*), and (*c*), dorsal, ventral, and lateral views respectively of the anterior region of the body of a *Desmognathus fusca* larva in which metamorphic changes have begun to appear; (*d*), (*e*), and (*f*), dorsal, ventral, and lateral views respectively of the anterior region of the body of a *Desmognathus fusca* in which the process of metamorphosis is just completed; *eyl*, eyelids, present only after metamorphosis; *gl*, gills which have become slightly blunted and shortened in (*a*), (*b*), and (*c*), and have disappeared altogether except for a slight vestige, *glv*, at the time of metamorphosis; *gr*, nasolabial groove, present only after metamorphosis; *gu*, gular fold; *lf*, labial fold which disappears at metamorphosis; *ori*, orifices of some of the larval acinous glands. Note that with metamorphosis the posterior region of the head becomes relatively narrower. Drawn with Abbé camera. $\times$ 4.25.

7. The labial folds (*lf*, Fig. 17) disappear, and the extent of the mouth opening increases, the lateral angles coming to lie as far back as the posterior angle of the orbit; thus a form of mouth opening adapted to the capture of larger prey, from a terrestrial as well as an aquatic medium, supplants the scooping or sucking apparatus of the aquatic larva.

8. The whole external surface gives evidence of the formation of a thin moult layer of epidermis, which here and there becomes separated from the deeper layers and thus presents a loose appearance.

In individuals in captivity these changes in external appearance have been observed to occur in the course of a few days during which time the individual crawls persistently out of the water. The metamorphosis may be considered quite complete when, in addition to the above enumerated changes in external appearance, the distinctive physiological metamorphic phenomenon occurs, and the animal, with mouth tightly closed, lowers the floor of the mouth and pharynx, and, drawing in air through the short nasal passages, fills its bucco-pharyngeal cavity and thus establishes aerial respiration.

Although the final steps in metamorphosis take place with great rapidity, the histological changes preliminary to the process begin considerably earlier. Thus specimens of larvæ collected in May show decided indications of the approaching change. The most striking of these indications is the large amount of mitosis which is in progress especially in the deeper layer of the epidermis (Plate V., 26, 27 and 29), resulting in so rapid a multiplication of the epidermal cells that the earlier arrangement in two fairly definite layers gives place to a considerable irregularity of arrangement of the cells of the deeper layer, the epidermis thus coming practically to consist of three layers. The arrangement of the cells of the outer layer remains unaltered, however, their cuticular borders forming, as before, a continuous external covering. The cells of the outer layer become, however, more and more flattened and are eventually to be cast off as the first moult layer, not, however, until after metamorphosis.

This flattening of the external layer of cells in part accounts for the actual decrease in thickness of the epidermis which becomes evident as the time of metamorphosis approaches; by far the greater cause of this, however, lies in the gradual disappearance of the Leydig cells, which by their turgor so greatly increase the thickness of the epidermis during early larval life. The invasion of these cells by pigment cells, connective tissue

cells and leukocytes during the larval period has already been noted. As metamorphosis approaches (Plate V., 28, 29, and 30), the nuclei of the Leydig cells (*lc*) become shrunken and the cell space is finally entirely given over to the intruding elements (*pgc*(*inv*) and *leu*) from the deeper region. The surrounding cells of the deeper epidermal layer, although increased in number by mitosis so as to form practically two layers instead of one, are correspondingly decreased in size and thus do not together equal in thickness the former diameter of the Leydig cells.

The thickness of the whole skin, *i. e.*, epidermis plus corium, does not become less; for coincident with the decrease in thickness of the epidermis there is a formation of loose vascular corium (*cor.l*) external to the dense corium and separating from it the deeper layer of epidermal cells which are in contact with it during the larval period. The formation of this loose corium is indeed inaugurated in the migration of leukocytes and connective tissue elements into the vacuoles of the Leydig cells and between the cells of the epidermis. But it is only with the breaking up of these Leydig cells in preparation for metamorphosis that these elements and other similar ones come to form a definite layer, and even then many of the connective tissue elements intrude themselves between the epidermal cells.

The development of integumental glands is particularly characteristic of the preparation for metamorphosis. These glands may be enumerated as follows: (1) General acinous glands; (2) naso-labial glands; (3) orbital glands.

The acinous gland anlagen appear during the general premetamorphic mitotic period of the epidermis (Plate V., 29 and 30). They arise from the deeper layer of the epidermis and may first be distinguished as little groups of from four to eight cells with large nuclei, some of which are usually in the process of mitosis (*ac*, Plate V., 29). They are abundantly distributed over practically all regions of the body, although somewhat less numerous in the skin covering the appendages. They rapidly increase in size and assume the form of hollow spherical acini, each with a definite lumen which opens to the exterior by a narrow duct (Plate V., 30). The bodies of the glands intrude into the loose corium and thus lie below the level of the epidermis,

which is, however, thin in the region immediately surrounding the gland. Embedded thus in the loose vascular corium, each gland comes to be surrounded by a capillary ring (*cap*), which, joining similar rings about the neighboring glands, forms a part of the rich capillary network of the skin. These glands and their ducts, the latter slender and lined with thin cells which are continuous with the cells of the external layer, are fully formed at the time of metamorphosis and before the first moult (*ml*, Plate VI., 31) occurs. The nature and function of their secretion and the methods by which they are replaced must be left for further and more extended investigation. Suffice it to say that many, if not all of them, show from the first a striking similarity in structure to the relatively larger and less numerous acinous larval glands above described. One anatomical distinction between the larval and adult glands must, however, be clearly understood, namely, that the larval glands lie wholly *below* the level of the dense corium, the ducts alone passing through this to reach the external surface. The larval glands remain functional up to the time of metamorphosis, and thus in the metamorphosing larva both the larval and the adult sets of glands are present, the former beneath the dense corium, the latter external to it within the loose corium (cf. Fig. 15, *e*). After metamorphosis, however, the larval glands collapse and lose their connection with the external surface, their cells undergo atrophy, and the glands rapidly disappear. It is this atrophy of the supra-branchial group of glands which results in the marked narrowing of this region of the head at metamorphosis (cf. Fig. 17).

The naso-labial glands consist of a series of tubular glands opening in close proximity to the external nasal orifices and along the border of the naso-labial groove (Whipple, '06). Of these glands (many of which become very extensive in the fully developed adults) the two largest (nl_1 and nl_2, Figs. 18, 19, and 20) begin their development before metamorphosis, and at the time of metamorphosis have attained a size and condition indicative of their functional importance in adult life. The one of these which opens at the dorsal angle of the nasal orifice extends, at this time, beneath the skin as far posteriorly as the anterior angle of the orbit, is somewhat convoluted, and branches near

its distal (posterior) end; the other gland, which opens near the ventral angle of the naris, is also well developed, extends nearly as far posteriorly as the first but in a more ventral location, and as yet shows no branching. Although other glands of the

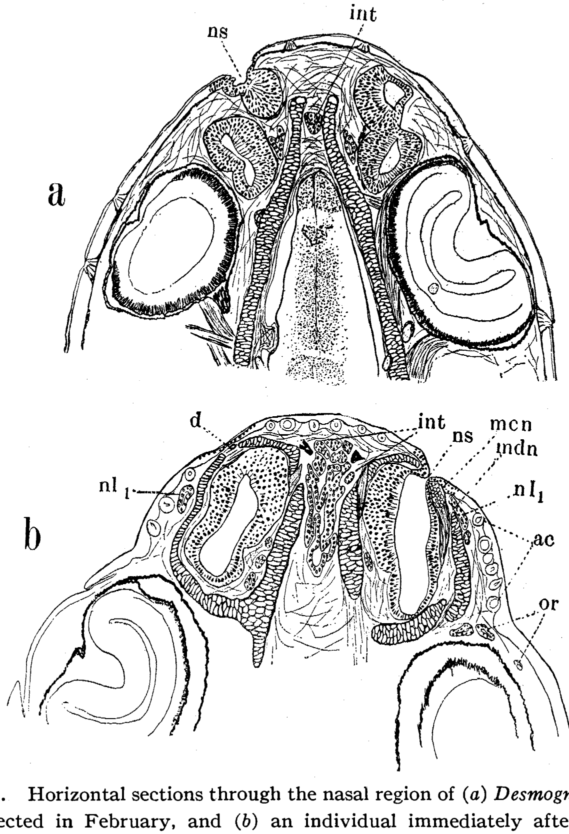


FIG. 18. Horizontal sections through the nasal region of (*a*) *Desmognathus fusca* larva collected in February, and (*b*) an individual immediately after the completion of its metamorphosis, for comparison of the glandular development; *ac*, acinous glands of adult stage; *d*, duct of the most dorsal of the naso-labial glands (nl_1); *int*, intermaxillary glands; *mcn*, and *mdn*, musculus constrictor naris, and musculus dilatator naris respectively; nl_1, the first nasolabial gland; *ns*, external naris; *or*, orbital glands. Drawn with Abbé camera. × 32.5.

group are slightly developed, none of the tubules have as yet become so extensive as to enter either the premaxillary foramina or the grooves of the maxillary bones as they are to do later in the fully developed adult. Simultaneously with the development of these naso-labial glands the muscular apparatus for opening

and closing the naris develops. This consists of a constrictor and a dilatator muscle (*mcn* and *mdn*) and forms an effective apparatus very characteristic of adult amphibians (Bruner, '96 and '01, I. W. Wilder, '09).

The naso-labial glands have to do with the terrestrial life into which the process of metamorphosis introduces the animal, in that they not only keep pliable the thin crescentic fold which by means of the contraction of the constrictor muscle closes the external naris against the entrance of foreign bodies, such as dirt when the animal is burrowing, but they also appear to have the peculiar

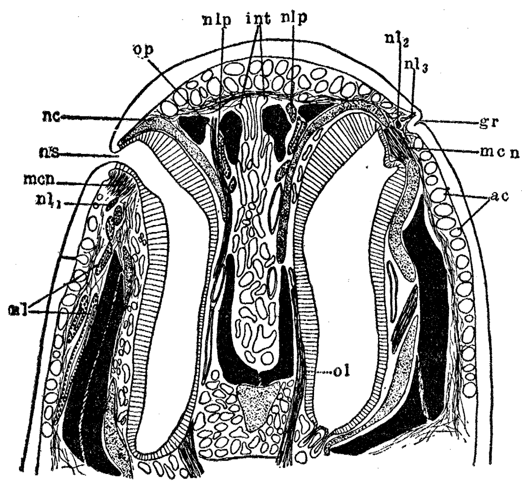


FIG. 19. Horizontal section through head of adult *Desmognathus fusca*, × 20. *Ac*, acinous glands of skin; *gr*, cross-section of naso-labial groove; *int*, intermaxillary glands; *mcn*, M. constrictor naris; *nc*, nasal capsule; *nl*, naso-labial glands; nl_1, nl_2, nl_3, the first, second and third naso-labial glands respectively; *nlp*, tubules of naso-labial glands within the premaxillary foramina; *ns*, external naris; *ol*, olfactory nerve; *op*, internal nasal branch of ophthalmic nerve.

function of so repelling water from the surface of the skin immediately surrounding the naris and bordering the naso-labial groove that the latter may, by capillary action, almost instantaneously drain off the tiny drop which fills the nasal depression after a temporary immersion in water. Thus this water is prevented from entering the nasal passage when the animal, upon emerging from the water, reopens the nares by withdrawal of the crescentic fold. The nasal passages are by this means kept dry and fully

ready for the immediate resumption of aerial respiration (Whipple, '06). It is a significant fact, therefore, that at the completion of metamorphosis this naso-labial apparatus is in working order.

The orbital glands lie ventral to the eyeball (*or*, Fig. 21, *b* and *d*)and open along the depression between the inner surface of the lower eyelid and the eyeball itself. They are tubular glands and make their appearance simultaneously with the formation of the fold which gives rise to the lower eyelid itself, shortly before metamorphosis.

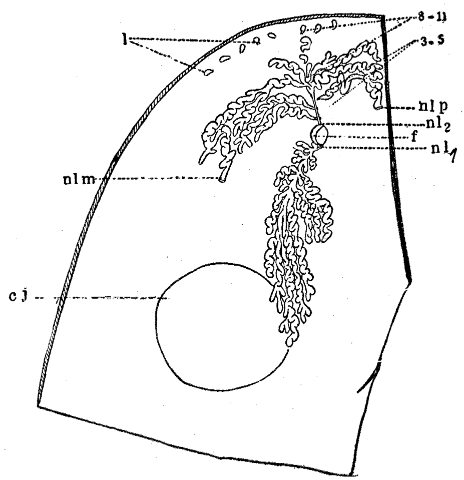


FIG. 20. Dissection of adult *Desmognathus fusca* in which the skin is removed from the right side of the head, the naso-labial glands being removed with it. Drawing shows under surface of skin with glands in place. *Cj*, conjunctiva; *l*, labial glands; 3–5 and 8–11 indicate the enumeration of the naso-labial glands; *nlm*, cut end of tubule which lies in the groove of the maxillary bone. Other designations as in Fig. 19.

In connection with the development of the orbital glands it should here be noted that unlike all other Urodeles (so far as the facts have been reported), *Desmognathus* has no naso-lacrimal ducts. As these are present in so closely related and associated forms as *Spelerpes* and *Plethodon*, as well as in the less closely related lunged forms, their absence here has some significance which demands further study of the comparative morphology and the habits of this species.

In addition to the integumental glands, the development of two other sets of glands must be noted among the premetamorphic changes, the intermaxillary and the lingual glands. The former is an unpaired gland and is present indeed throughout larval life (*int*, Fig. 18) as a single, slightly convoluted tube

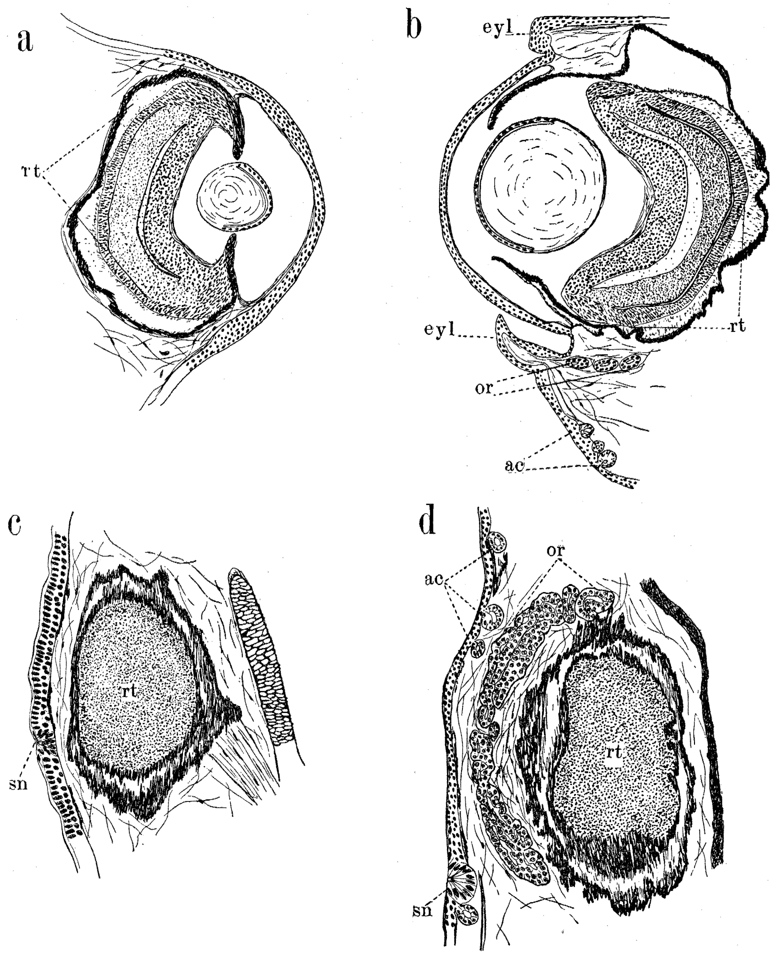


FIG. 21. Sections for comparison of the eye and its surroundings in the larval and adult stages of *Desmognathus fusca*; (*a*) transverse section through the left eye of a larva; (*b*) transverse section through the right eye of a recently metamorphosed adult; (*c*) horizontal section through the ventral region of the eye of the larva; (*d*) horizontal section through the ventral region of the eye of a recently metamorphosed adult. *Ac*, acinous glands; *eyl*, eyelids; *or*, orbital glands; *rt*, retina; *sn*, sense organs of the integument. Drawn with Abbé camera. × 60.

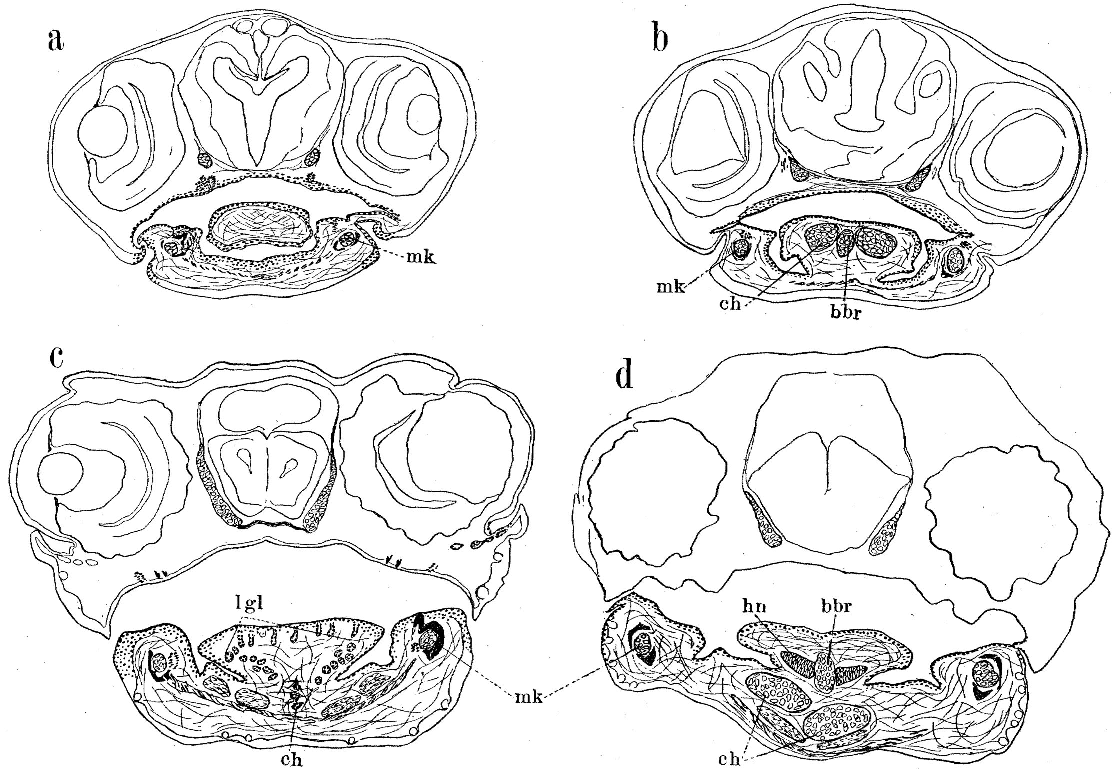


FIG. 22. Cross sections through the heads of (*a*) *Desmognathus fusca* larva at the level of the free anterior region of the tongue, (*b*) the same larva at the level of the articulation of the ceratohyals and basibranchial (cf. Fig. 25, *a*), (*c*) recently metamorphosed adult at a level corresponding to (*a*), and (*d*) the same adult at the level corresponding to (*b*) (cf. Fig. 25, *b*); *bbr*, basibranchial; *ch*, ceratohyal; *hn*, accessory horns; *lgl*, lingual glands; *mk*, Meckel's cartilage. Drawn with Abbé camera. × 32.5.

leading dorsally from the roof of the mouth, from a point a little anterior to the posterior nares. During the premetamorphic period of mitosis and glandular development, the intermaxillary gland becomes enormously enlarged and complicated and fills the space between the two maxillary bones with its mass of convoluted tubules.

The tongue possesses no multicellular glands until, with the approach of metamorphosis, large numbers of tubular glands (*lgl*, Fig. 22, *c*) appear opening in the more distal region of the tongue upon its dorsal surface, and transforming the tongue into a glandular mass. The development of both intermaxillary and lingual glands at this time is, of course, suggestive of a preparation for the terrestrial life of the adult. By means of their secretions the mouth is kept moist both for its respiratory function and to render more sure the capture and retention of terrestrial prey.

Among the most significant of the structural changes involved in metamorphosis are those concerned in the atrophy of the gills and the modifications of their associated structure, the visceral skeleton. Externally changes do not occur in the gill bushes themselves until within a few days of the completion of metamorphosis. Then the filaments become shorter and more rounded, and the whole structure undergoes rapid shrinkage and atrophy. As the gills are merely blood vessels covered with a thin layer of epidermic cells, the withdrawal of the blood from them so reduces them in size that the process of atrophy can take place very quickly.

The metamorphic changes in the visceral skeleton consist mainly in the atrophy of the distal end of the first epibranchial and the atrophy of the whole of the second, third, and fourth epibranchials (Figs. 23, 24, and 25). The process begins at the time of the premetamorphic mitotic period, and appears first in the distal ends of the epibranchial cartilages where the hyaline matrix becomes dissolved around the groups of cartilage cells (Fig. 23, *b*). With the atrophy of the free ends of the epibranchials, there occurs that shortening of the gill region which is mainly responsible for the decrease in the proportionate length of the head as the animal passes from larval to adult life. Simul-

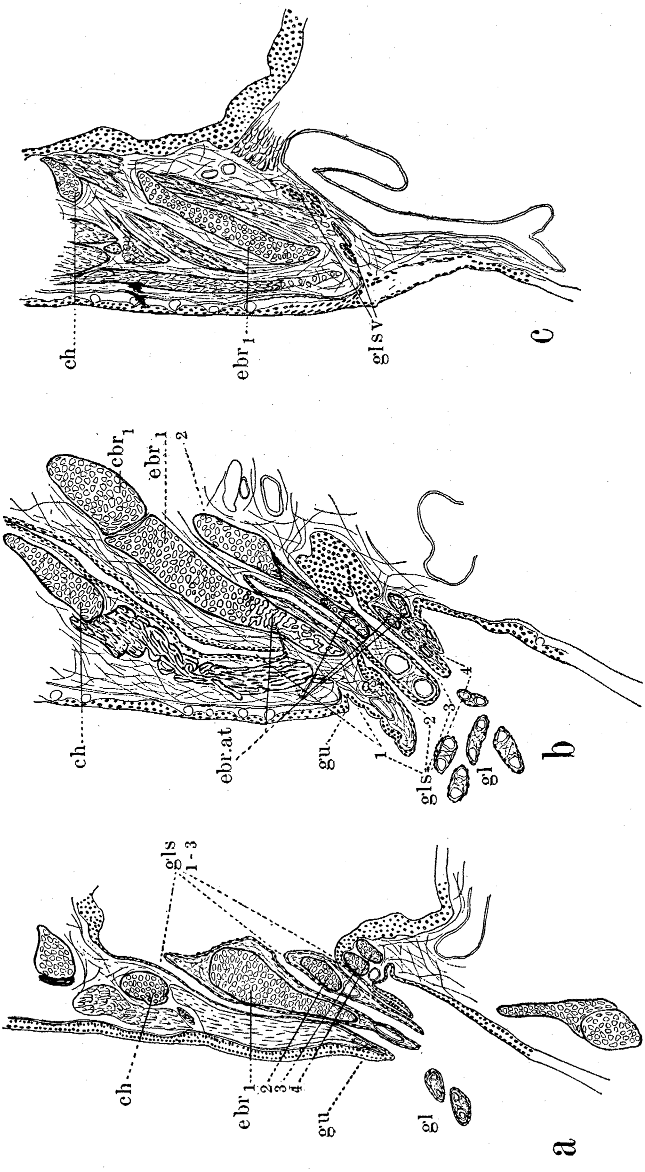


FIG. 23. Horizontal sections through the gill region of *Desmognathus fusca* illustrating the changes which take place at metamorphosis; (*a*) aquatic larva collected in February, showing the typical larval condition of the region; (*b*) larva in which metamorphic changes have begun (cf. Fig. 17, *a*, *b*, and *c*); (*c*) an individual which has just completed its metamorphosis (cf. Fig. 17, *d*, *e*, and *f*); *ch*, ceratohyal cartilage; *cbr*1, first ceratobranchial cartilage; *ebr 1, 2, 3,* and *4*, epibranchial cartilages; *ebr.at*, regions of the epibranchials which are undergoing atrophy; *gl*, gills; *gls*, gill slits; *glsv*, vestiges of the gill slits; *gu*, gular fold. Drawn with Abbé camera. ×32.5.

taneously with the atrophy of the epibranchials there occurs an atrophy of the whole of the second basibranchial except its extreme posterior end, which persists and later ossifies to become a little bifurcated structure designated by Wiedersheim ('77) as the thyreoid bone (*bbr 2*, Fig. 25, *b*). With the atrophy of the second basibranchial cartilage, which during larval life forms one continuous chondrification with the first pair of ceratobranchials, the latter become separated from each other and each articulated

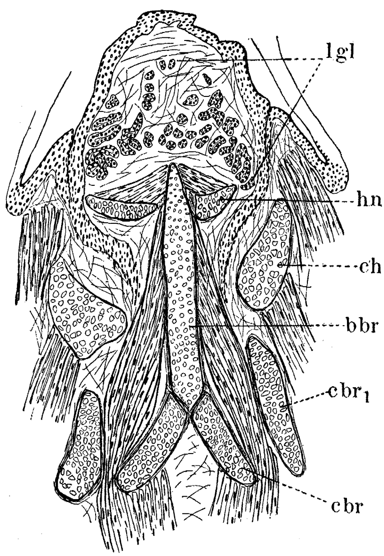


FIG. 24. Horizontal section through the tongue and hyo-branchial apparatus of a recently metamorphosed adult *Desmognathus fusca* (cf. with the drawing of the whole apparatus shown in Fig. 25, *b*); *bbr*, basibranchial; *cbr 1* and *2*, first and second ceratobranchials; *ch*, ceratohyal; *hn*, accessory horns for the support of the adult tongue; *lgl*, lingual glands. Drawn with Abbé camera. × 32.5.

with the first basibranchial. In the two or three final days before metamorphosis the medial ends of the ceratohyals become detached from the anterior ends of the first basibranchial (*ch*, Fig. 22, *b* and *d*), while posterior to their former point of attachment, a pair of cartilaginous processes grow out from the basibranchial to serve apparently as an additional support for the tongue (*hn*, Figs. 22, *d*, 24, and 25).

The final change which completes the atrophy of the gill region is the closure of the gill slits of which there are four upon

each side, the most anterior being the extensive one anterior to the first gill arch and beneath the gular fold. Previous to metamorphosis the gular fold becomes noticeably shortened, keeping pace with the shortening of the cartilaginous arches.

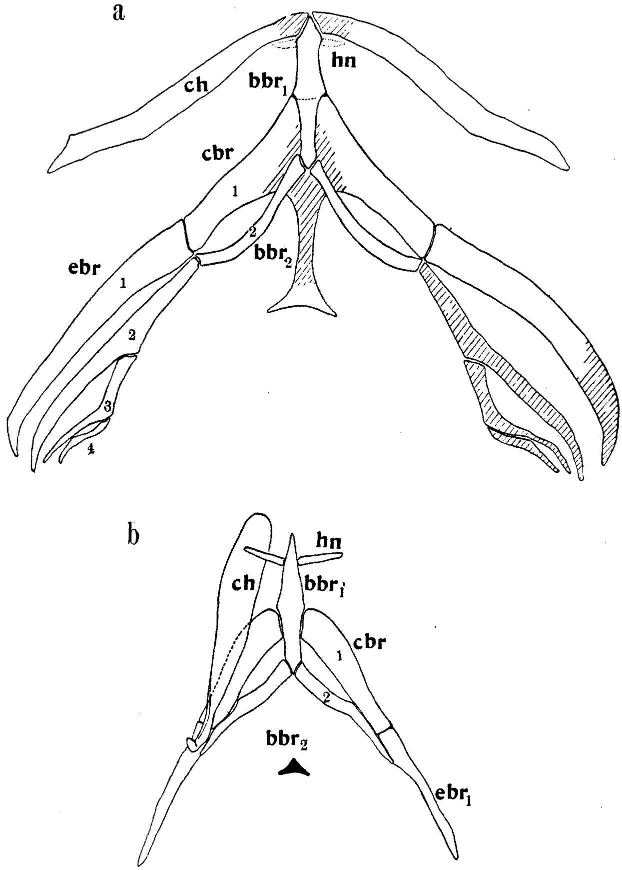


FIG. 25. (*a*) A dorsal view of the hyo-branchial apparatus of an aquatic larva collected in mid-winter, showing the typical larval structure. The shading indicates the regions which will atrophy at metamorphosis. The dotted lines indicate the regions where the accessory horns (*hn*) will develop at the time of metamorphosis. *Bbr_1* and *bbr_2*, first and second basibranchials; *cbr_1* and *cbr_2*, the first and second ceratobranchials; *ch*, ceratohyal; *ebr 1, 2, 3*, and *4*, the four epibranchials. Drawn with Abbé camera from a preparation stained *in situ* with methylene blue, and cleared with clove oil. × 40.

(*b*) A dorsal view of the hyo-branchial apparatus of an adult *Desmognathus fusca*. Designations as in (*a*). Note the persistence and ossification of the posterior end of the second basibranchial, forming the *os thereoideum* of Wiedersheim ('77). Drawn with Abbé camera from a dissection. × 6.

With the final withdrawal of the blood from the gill bushes, the latter lie against the lateral wall of the pharyngeal region like a temporary operculum, and rapidly fuse with the epidermis covering the surface over which they lie, thus effecting the external closure of the gill slits (Fig. 23, *b* and *c*).

For some time after metamorphosis the whole region is in a disorganized condition histologically, and sections show beneath an external covering of the thinnest of epidermic layers, a great meshwork of distended blood vessels, together with the remnants of epithelial lining of gill slits, which through the agency of the ever active leukocytes is rapidly disintegrating.

The transition from larval to adult life involves in *Desmognathus* no fundamental change in the nature of the food, since throughout the whole life of the species the food consists mainly of living animal forms. As would be expected, therefore, metamorphosis necessitates no noticeable changes in the digestive apparatus, as it does in the case of those amphibians in which the nature of the food changes at metamorphosis from vegetable to animal.

Adult Stage.

With regard to the growth and development of *Desmognathus* subsequent to metamorphosis I can give no very exact data. The many adults which I have measured range from 29 mm. to 104 mm. in length, the former showing, of course, unmistakable evidences of recent metamorphosis. Specimens collected at any one time do not show sufficient evidence of falling into distinct groups as to size to suggest definite yearly amounts of growth, with the possible exception of the smaller sizes which, in the case of those collected in the late spring, for example, fall into groups averaging 33 mm., 43 mm., and 56 mm. in length, the former recently metamorphosed, and the others presumably adults of one and two years' growth, respectively. Since the proportionate lengths of body regions of the smallest adults show a much wider range of variation as well as a closer similarity to the proportions of the larvæ than to those of adults of 55 mm. and over, it seems evident that not only is the growth during the first year or two more rapid but that the change of length pro-

portions from those at metamorphosis to those of the final adult life, a change which involves a relatively more rapid growth of the caudal than of the other regions, also takes place more rapidly during the first two years (cf. Table II., Graphs III. and IV.). All of the adults, however, show a slight range of variation in the proportions of length of body regions which is in general correlated with the size of the specimens, as if the caudal region continued always to grow slightly more rapidly than the rest of the body. Here, however, the actual facts are sometimes masked by the frequency of regeneration of the tail, since a large percentage of adults show the tail to be in some stage of regeneration. I have seen no evidence that this loss of tail is due to self-mutilation as in the case of *Plethodon*, although the natural supposition would be that such is the case. Such regenerating specimens have, when detected, been excluded from my statistics, but when the process of tail regeneration is nearly complete, it is not always possible to decide whether the specimen presents a case of regeneration or one at the lower limit of normal variation. The question of the time of sexual maturity may also throw some light on the duration of the period of the more rapid growth of adults. At the time of metamorphosis the two sexes may be distinguished by the histological condition of the germ cells, some of the ova having already entered upon the growth period. Small adults, up to the 43 mm. stage, *i. e.*, presumably of a year's growth as adults, give no indication of sexual maturity. I have had too little opportunity to examine in this particular, specimens of the next larger size, presumably of two years' growth, to be able to state definitely that they may not be sexually mature at this time, but the few that I have examined were not so. On the other hand, specimens of 68 mm. and over of both sexes are sexually mature, apparently after three years of adult life. In the mating season the lips of the cloaca are often somewhat everted, and the two sexes may be distinguished by the fact that the female cloacal lips display numerous folds which converge anteriorly, with an anteriorly projecting papilla in the mid-line at the posterior angle of the two lips. In the end of this papilla, which lies within the cloacal cavity, is the orifice of the spermatheca. The lips of the male cloaca are thickly covered with villi-like processes.

The changes in structure during adult life are otherwise those of the natural growth of the various organs. Thus, although the epidermis retains the condition reached soon after metamorphosis of some two or three layers of cells beneath the moult layer, the acinous glands increase constantly in size and number, and the loose corium in which they are embedded, as well as the dense corium beneath, becomes correspondingly thicker and the whole skin more deeply pigmented and more vascular. So also the other integumental glands, such as the naso-labial and the orbital, become more numerous and their tubules more convoluted and longer, while the naso-labial groove itself grows deeper and more clearly marked.

The muscular development keeps pace with the general increase in size. Thus the power and rapidity of motion which may be observed in all stages of this species becomes very pronounced in the large adults. The long muscular tail plays an important rôle in locomotion, acting as a strong propelling organ as it strikes against the ground first upon one side, then upon the other, thus sending the body forward. This method of locomotion is of course a part of the adaptation to a burrowing habit.

The following list of the contents of the stomachs of 18 specimens of adult *Desmognathus* of different sizes and collected at different times and from different localities will serve to illustrate the variety of food materials which are made use of. It will be noted that the food is wholly animal and that the list includes aquatic as well as terrestrial forms.

Specimen No. 1. Moulted skin of *Desmognathus* (presumably his own).

No. 2. A caddice fly, and three dipterous larvæ.

No. 3. A small adult dipterous insect.

No. 4. Unidentifiable animal fragments.

No. 5. Insect larva, unidentified.

No. 6. Spider, probably an *Azalena*, with egg mass.

No. 7. Large black ant.

No. 8. Fragments of beetles, hymenopterous insects, and sowbugs.

No. 9. Two small beetles, a dipterous insect, a small green caterpillar, and an insect larva.

No. 10. Remains of an aquatic insect larva.

No. 11. Sand, dirt, and unrecognizable debris.

No. 12. A small annelid, fragments of a larger annelid, two small crustaceans (probably *Gammarus*), some mites, and fragments of adult insects.

No. 13. An adult dipterous insect, fragments of other small insects, some very minute mites, and unrecognizable debris.

No. 14. A small mite.

No. 15. A whole *Desmognathus* larva.

No. 16. A whole *Desmognathus* larva.

No. 17. Moulted skin of *Desmognathus*.

No. 18. An earthworm, a small spider, and some six or eight specimens of *Gammarus*.

The breathing habits of adult *Desmognathus* deserve especial discussion because of the lungless condition of the animal. As in all amphibians the skin is a most efficient breathing organ, and so long as it is kept in the normal moist condition which the burrowing habit of the species insures, it probably furnishes ample means for the aeration of the blood which circulates through its capillary network. As is usual with amphibians also, the bucco-pharyngeal cavity is made use of to supplement the cutaneous respiration. Bucco-pharyngeal respiration is accomplished by a rapid fluctuation of the floor of the tightly closed mouth, which results in a rapid succession of movements of air in and out through the wide-opened nares. Correlated probably with the lungless condition, is the fact that these fluctuations are far more rapid in *Desmognathus* than in the lunged forms; moreover, *Desmognathus* in common with other lungless forms (*Spelerpes* and *Plethodon* for example) possesses a relatively longer œsophagus provided with muscles by means of which it may be held distended, thus increasing the size of the respiratory surface by the addition of a mucous membrane which was shown by the researches of H. H. Wilder ('01) and Seelye ('06)

to be supplied with a rich capillary network, rendering it an efficient breathing organ.

As I have shown elsewhere (Whipple, '06), the lungless salamanders do not have the habit, otherwise common to amphibians, of changing from aerial to aquatic bucco-pharyngeal respiration when in the water, even though they may be kept completely immersed for several days. Under these conditions the external nares are kept tightly closed and the floor of the mouth forcibly drawn in. The immersed condition is of course an unnatural one for *Desmognathus* as is shown by its frantic efforts to escape from the water, but it shows nevertheless no physical ill effects of a prolonged imprisonment beneath the surface, a proof that the skin is under these conditions a perfectly adequate breathing organ.

In his paper on *Desmognathus fusca* and *Spelerpes bilineatus*, H. H. Wilder ('99) pointed out the great possibilities which are presented by both *Desmognathus* and *Spelerpes* as objects for laboratory study. The abundance and widespread occurrence of the two genera, the ease with which they may be collected in both the larval and the adult states throughout the entire college year, and the convenience with which they may be kept alive in the laboratory for months, with practically no care, render the material always available and inexpensive. The following account taken from Wilder ('99) gives valuable suggestions as to methods of keeping such material in the laboratory:

"*Method of Rearing in Confinement.*—The adults of both species, because of their peculiarities in respiration and the consequent necessity of keeping their skin moist, cannot be kept either in water or in a dry atmosphere, but may easily be kept for months or years in an ordinary fernery where the atmosphere is constantly saturated with moisture. I have in my laboratory a large fernery or terrarium, about 2 × 3 feet square and 2 feet high. The bottom consists of a zinc tray, 8 inches deep and water-tight. The top and sides are of glass and the front side runs in a frame with weights, being thus capable of being raised and lowered like an ordinary window-sash. In the bottom of this there are about 6 inches of good garden soil, in which are planted ferns and other wood plants. The surface is partly

covered with moss, and here and there are placed several stones, the size of one's fist, and a few pieces of rotten stump, arranged so as to give shelter to the adults. In one corner a crystallizing dish is sunk to the level of the soil. This is filled with water and the bottom covered with a little fine sand. Some duckweed, or *Salvinia*, may be placed upon the surface, and a few small stones should be put in a dish. At the beginning of the season, after arranging everything as above, enough water is poured in to drench the soil, and the sunken dish is filled. After this the terrarium is self-regulating. The water that evaporates is re-precipitated as moisture, and the total loss from the little pond in the corner is so slight that it needs replenishing not oftener than once in six months. If the terrarium is to support many animals, it is better to place a few earthworms, myriapods, etc., in it; and if the pond is designed for the rearing of larvæ, supplies of *Entomostraca* and a little *Spirogyra* to feed them with should be occasionally introduced. I have tried placing tiny bits of meat in prominent places, but they merely mould and have to be removed. I have kept as many as 20–30 adults and a dozen larvæ in my terrarium during an entire college year, and several times, on clearing it out in the fall after the summer vacation, I have found alive and in good condition adults which I had been unable to find in the spring, when I intend always to remove the animals. It seems most probable that these salamanders find enough to eat among the worms and insects introduced with the earth and plants, as they always appear in perfectly normal condition and contrast very forcibly with *Diemyctylus*, which grows thin and often starves to death when placed under the same conditions."

I might add to this account the fact that I have found very convenient for the accommodation of a small number of specimens, such terraria as may be readily constructed from cylindrical anatomical jars of heavy glass, measuring from 8 to 18 inches in diameter and from 6 to 10 inches high, and provided with a tightly fitting but easily removable glass cover. Such jars, equipped with earth, stones, and plants as above directed by Wilder, will accommodate according to size from two to ten specimens of adult *Desmognathus* and are small enough to be

easily moved about. Moreover, owing to the fact that the adult *Desmognathus* occasionally feeds upon the larvæ, I have found it safer for the latter to be kept in separate shallow receptacles, such as glass crystallizing dishes, equipped with a little earth covered with water to the depth of a few centimeters, and containing a quantity of decaying, slime-covered leaves which have been transported to it from the natural habitat of the larvæ. Such an arrangement, if kept tightly covered to prevent evaporation, will support the larvæ in a normal condition for months without further care.

The usefulness of *Desmognathus* as material for the various phases of introductory research work cannot be over-emphasized. As Wilder has pointed out, the large size and the unpigmented nature of the egg render it excellent material for the study of general amphibian embryology. The small size of the larvæ (from 15 to 30 mm. in length), and their long continuance in the larval state, make them particularly valuable material for the introduction to methods of study by serial sections, since the specimens are not only small enought to decalcify easily and section beautifully but are large enough and enough like the adult in their general anatomy to also lend themselves readily to study by dissection, and by the numerous methods of injecting and selective staining followed by clearing *in toto*. Studied in this way it furnishes, pedagogically, an excellent means of transition from the study of the gross anatomy of larger vertebrate forms to the study both of their histology and their earlier embryology. Thus it bridges a gap which it has often been customary in college courses to make at a jump which too often lands even the able student in a region of mystery. As material for the study of adult anatomy, also, *Desmognathus* is a convenient form, the larger specimens being quite large enough for ready dissection, and not too large to use with the dissecting microscope for finer details, while both the large and the small adults may be readily decalcified and sectioned for the microscopic study of their structure. Moreover the species with its succession of terrestrial and aquatic stages, its lungless condition and other peculiarities of structure, presents a number of little physiological and anatomical problems, the working out of which

is not too difficult for the inexperienced beginner, and may even yield important contributions to our general knowledge.

It is with the hope, therefore, that these pages will be useful and suggestive to those interested in any amphibian problems upon which the study of *Desmognathus* might have some bearing, that it has seemed worth while to collate the known facts concerning the life history of this common and interesting species.

SMITH COLLEGE, NORTHAMPTON, MASS.,
June 26, 1912.

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List of Abbreviations Used in the Plates.

ac, acinous glands.
cap, capillaries.
cl, columnar epidermal cells.
cor.d, dense corium.
cor.l, loose corium.
cu, cuticle formed by the outer borders of the outer layer of epidermal cells.
d, duct of acinous gland.
ep, epidermis.
ext, outer layer of epidermal cells.

gc, giant cells of the acinous glands.

int, inner layer of epidermal cells.

intr, intrusive cells (Schaltzelle), which project from the deeper layer into the outer layer.

lc, Leydig cells.

leu, leukocytes.

lu, lumen of gland.

ml, moult layer.

mt, mitosis.

mt(*inv*), mitosis in a cell which has invaded a Leydig cell.

pg, pigment.

pgbr, branches of pigment cells.

pgc, pigment cells.

pgc(*inv*), pigment cells which have invaded the Leydig cells.

pgg, intracellular pigment granules.

sn, integumental sense organ.

sp, spireme.

yg, yolk granules.

Description of Plates.

Plates I., II., and III. A series of outlines drawn with Abbé camera, magnification × 4.25, showing various stages of *Desmognathus fusca* from the time of hatching to the attainment of practically adult proportions.

Plates IV., V., and VI. Sections of the skin of *Desmognathus fusca*, showing the structures characteristic of the various periods and the developmental changes which take place in the transition from one stage to another. Drawn with Abbé camera. × 450.

Errata.

References throughout the text to Pl. I., Fig. 7–11, apply to Pl. II.

PLATE I.

FIGS. 1 and 2. Lateral and ventral views of terrestrial larval stage *A*, immediately after hatching. Lines *7a*, *10b*, and *12a* show the locations of the sections shown in the text figures correspondingly numbered.

FIGS. 3 and 4. Lateral and ventral views of terrestrial larval stage *B*, age 3 days.

FIGS. 5 and 6. Lateral and ventral views of terrestrial larval stage *C*, age 3 days. Lines *10c* and *12b* show the locations of the sections shown in the text figures correspondingly numbered.

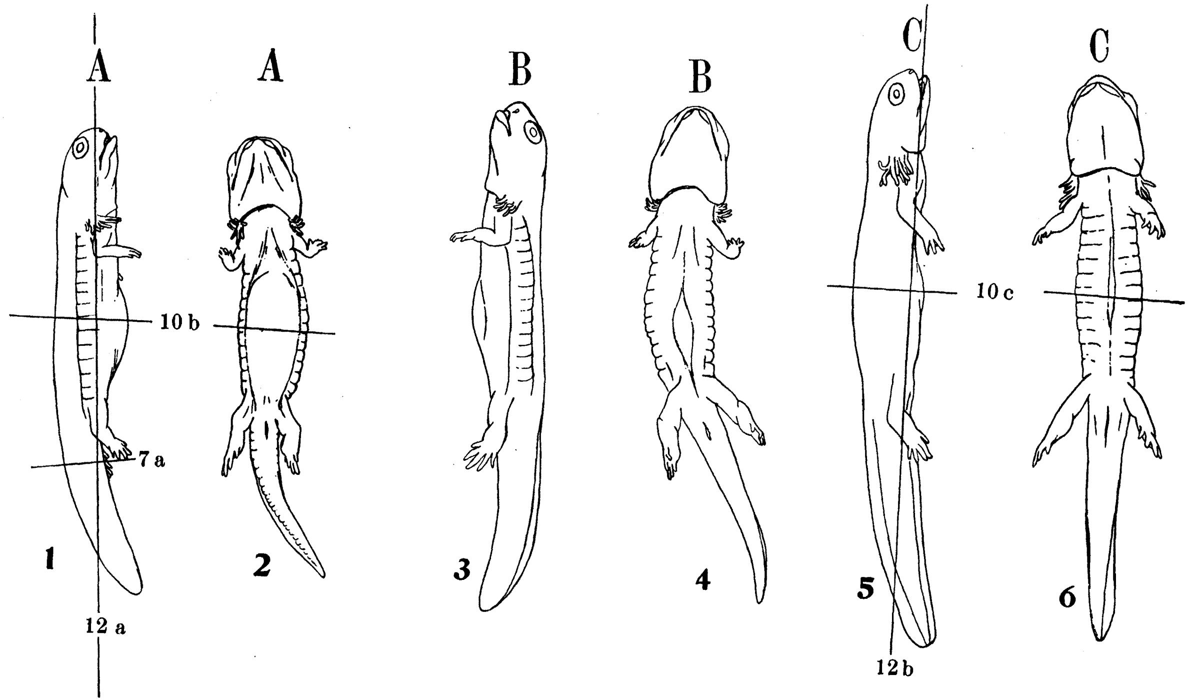

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PLATE II.

FIGS. 7 and 8. Lateral and ventral views of terrestrial larval stage *D*, age 7½ days.

FIG. 9. Lateral view of terrestrial larval stage *F*, a small specimen which has attained the proportions of the aquatic larvæ, age 15½ days. Line *10d* and *12c* show the location of the sections shown in the text figures correspondingly numbered.

FIGS. 10 and 11. Lateral and ventral views of an aquatic larva collected in October. Line *7b* indicates the location of the section shown in the text figure correspondingly numbered. The dotted lines show the levels used in arbitrarily dividing the body into head, trunk, and tail regions for measurements (cf. Tables I., and II., and Graphs I., II., III., and IV.).

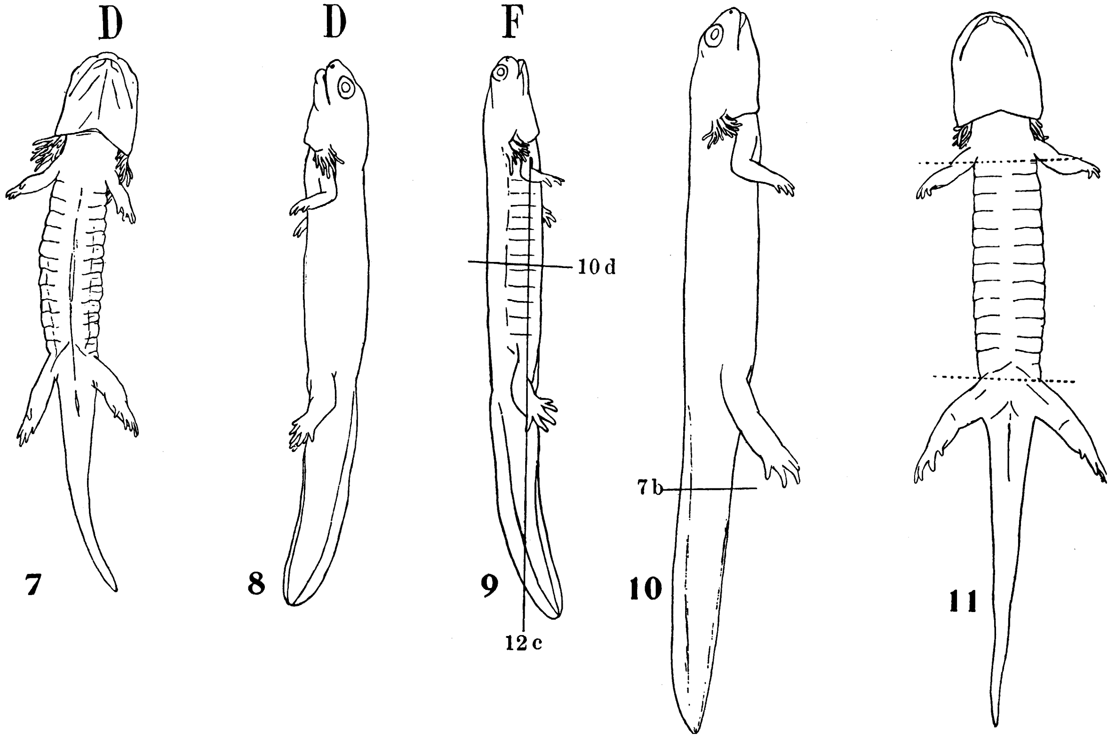

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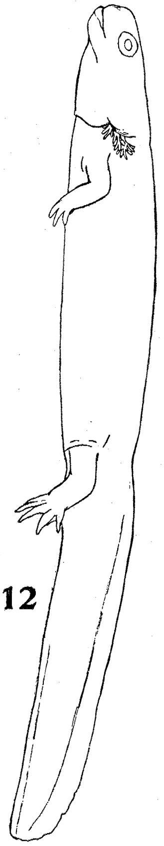
PLATE III.

FIGS. 12 and 13. Lateral and dorsal views of an aquatic larva collected in May, and hence nearly ready to undergo metamorphosis.

FIGS. 14 and 15. Lateral and dorsal views of a small, recently metamorphosed adult, collected in June.

FIG. 16. Ventral view of an adult 49.4 mm. long.



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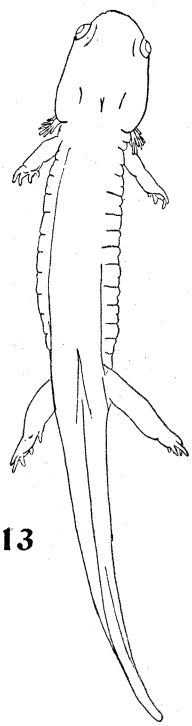

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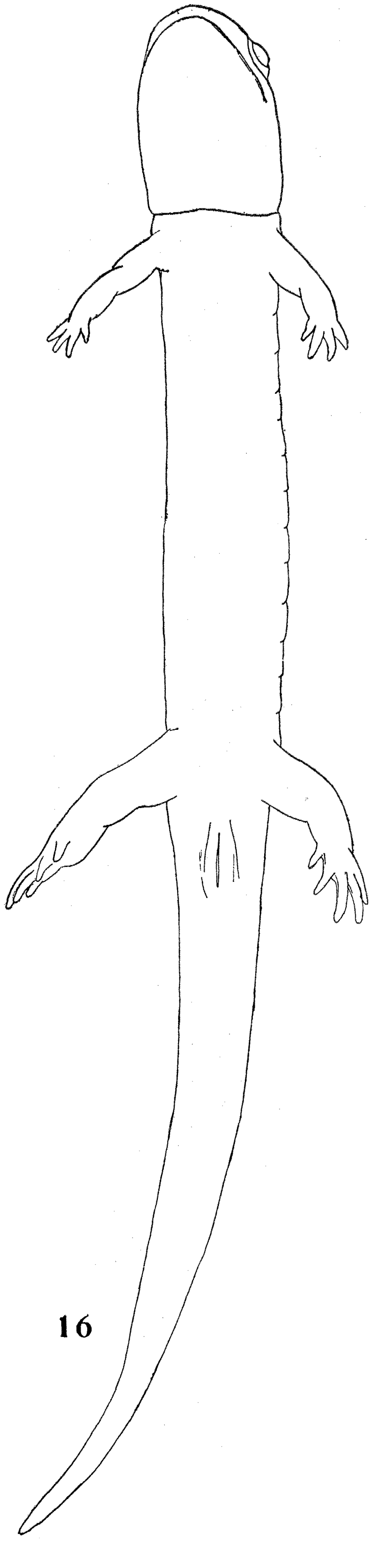

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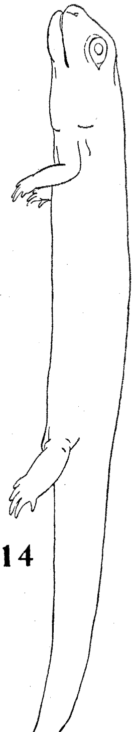

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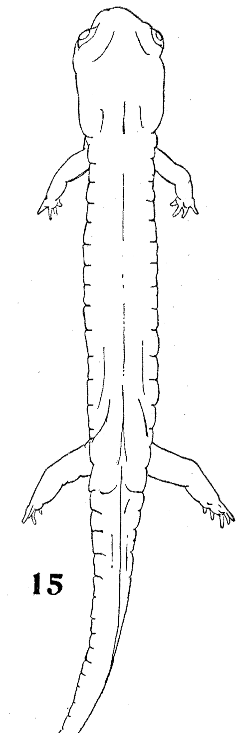

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PLATE IV.

FIG. 17. Vertical section through the skin of the dorsal surface of a 13 mm. embryo of 30 days' development, showing the characteristic two layers of epidermal cells.

FIG. 18. Vertical section through the skin of the lateral surface of a 13 mm. embryo.

FIG. 19. Vertical section through the skin of the dorsal surface of a newly hatched terrestrial larva, stage *A*. Note the presence of a corium of dense connective tissue beneath the two layers of epidermal cells, the absence of mitosis in the epidermis, and the development of an external cuticle from the outer borders of the outer layer of cells.

FIG. 20. Vertical section through one of the integumental sense organs in the region of the gular fold, terrestrial larva, stage *A*.

FIG. 21. Section parallel with the external surface, through the outer layer of epidermal cells, dorso-lateral surface, terrestrial larva, stage *A*.

FIG. 22. Similar section through the inner layer of epidermal cells, dorso-lateral surface, terrestrial larva, stage *A*.

FIG. 23. Vertical section through the skin of the dorso-lateral surface of an aquatic larva collected in September. Note the presence of numerous Leydig cells in the deeper layer.

FIG. 24. Section parallel with the external surface, through the outer layer of epidermal cells from a lateral, unpigmented region of the body of an aquatic larva collected in September.

FIG. 25. Similar section through the inner layer of epidermal cells, lateral region, of an aquatic larva collected in September.

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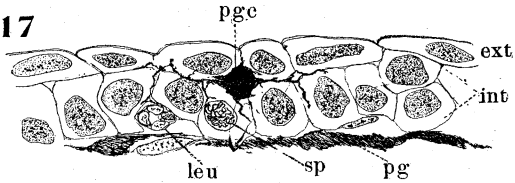

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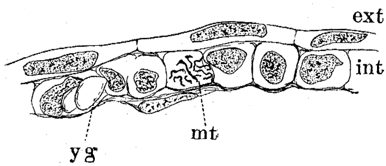

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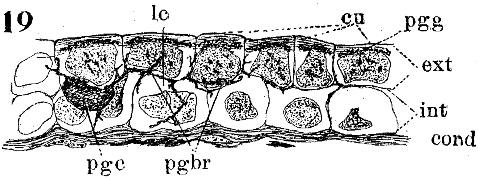

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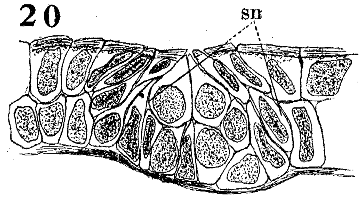

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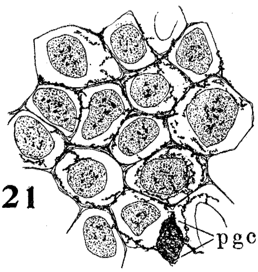

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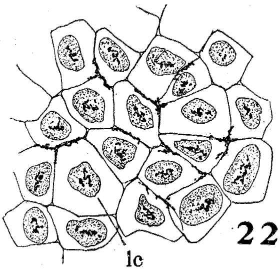

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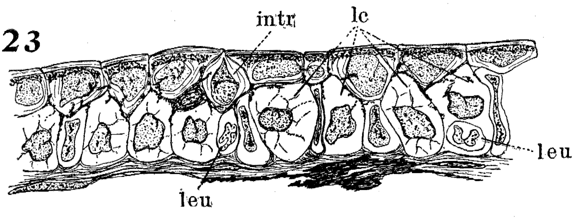

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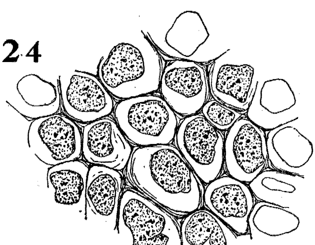

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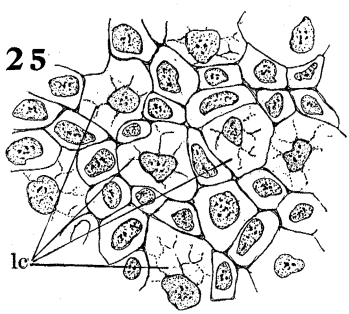

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PLATE V.

FIG. 26. Vertical section through the skin of the lateral region of the body of an aquatic larva collected in May and hence in preparation for metamorphosis as is shown by the presence of mitotic figures, and by the invasion of leukocytes.

FIG. 27. Section parallel with the external surface, through the outer layer of epidermal cells of the skin of an aquatic larva collected in May.

FIG. 28. Similar section through the inner layer of epidermal cells, aquatic larva collected in May.

FIG. 29. Vertical section through the skin of the ventral surface of the leg of an aquatic larva collected in May and showing the rapid progress of metamorphic changes, among them the increase in the number of cells by mitotic division, the development of adult acinous glands, the invasion of leukocytes, the atrophy of the Leydig cells, and the formation of the loose corium.

FIG. 30. Vertical section through the skin of a larva collected in June in which the metamorphic changes in the skin are nearly completed. Note, in addition to the features indicated in Fig. 29, the presence of a capillary network in the loose corium surrounding the acinous gland.

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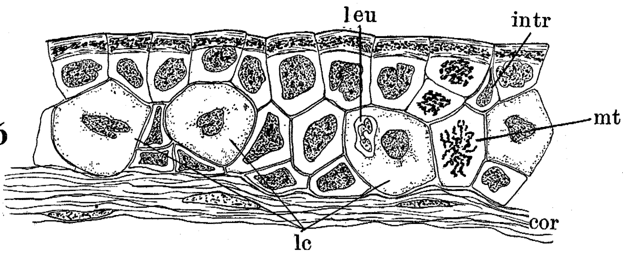
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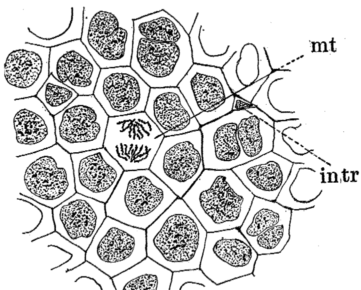
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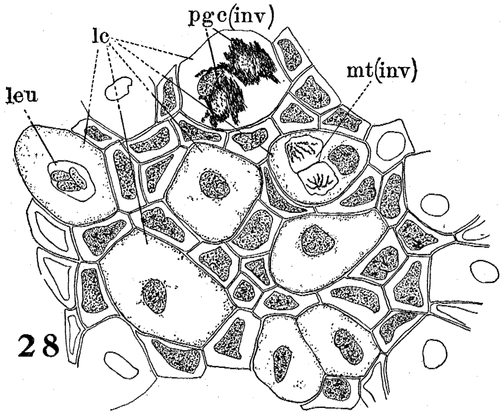
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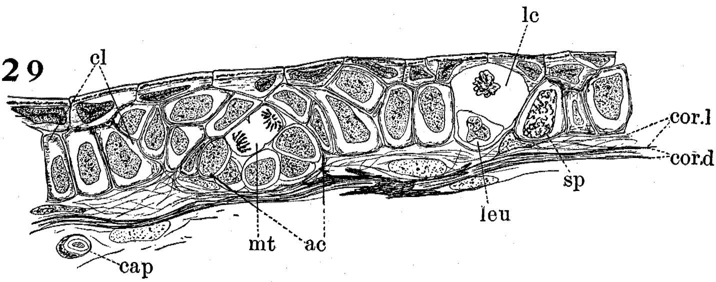
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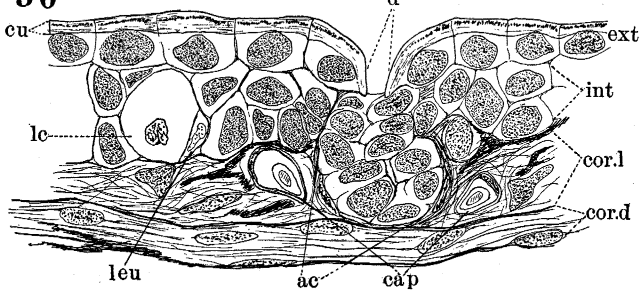
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PLATE VI.

FIG. 31. Vertical section through the skin of an individual which has just completed its metamorphosis, but in which the first moult has not yet occurred. Note the presence of a deeper layer of flattened cells which will become the next moult layer.

FIG. 32. Vertical section through the skin of a very small adult (length 29 mm.) collected in June shortly after metamorphosis and after the first moult has been cast.

FIG. 33. Vertical section through the skin of a large adult, showing the large size of the acinous glands, the great thickness of the loose corium, and the relative thinness of the epidermic layer (cf. larval condition).

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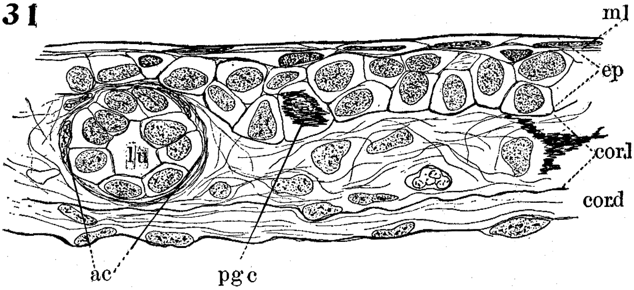
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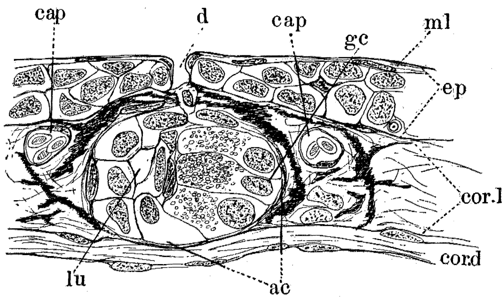
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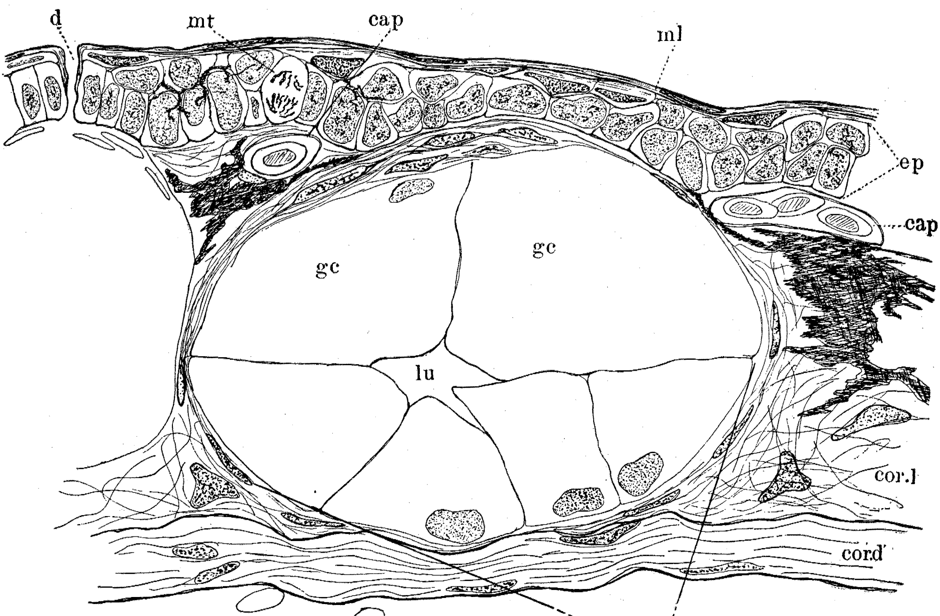
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